Supplemental Material

Supplemental Figure 1: Somatic point mutations in genes in the human HH signaling pathway as listed in the KEGG database that were found in the 48 early-stage and advanced BBCs. Genes are listed in order of descending number of mutations and the numbers on the far right and bottom indicate row and column totals.

Supplemental Table 1: Comparison of mean exonic mutations, HH pathway mutations, transitions, and SMO, TP53, and PTCH1 mutations in KNSTRN-mutated advanced BCCs and KNSTRN-wildtype advanced BCCs.

Supplemental Methods:

Tumor Samples and Sequencing

After Stanford Human Subjects panel approval, written informed consent was obtained from patients for tumor sequencing (Protocol 18325). Fresh tissue samples of 47 BCCs and normal skin pairs were obtained (one sample, BCC48, did not have paired normal skin, and so a second patient-matched tumor and the reference human genome were used to identify somatic mutations in KNSTRN; the paired tumor lacked a KNSTRN mutation, proving that the KNSTRN mutation in BCC48 was somatic. However, total number of exonic mutations, percent transitions, and CNV analysis for BCC48 could not be calculated due to the lack of a paired normal skin sample). DNA was isolated using the DNeasy Blood & Tissue kit according to manufacturer's protocols (Qiagen), capture libraries were constructed using the Agilent

SureSelect XT Human All Exon V4 kit according to manufacturer's specifications, and enriched exome libraries were multiplexed and sequenced on the Illumina HiSeq 2500 platform to generate 100-bp paired-end reads.

SNV and CNV Analysis

Sequencing data were mapped to the hg19 reference human genome sequence using the Burrows-Wheeler Aligner (BWA). Picard tools (version 1.86) was used for SAM to BAM conversion and marking of PCR duplicates, and Genome Analysis Toolkit (GATK) (v2.3.9) was used for indel realignment and base quality score recalibration prior to variant calling using both Samtools mpileup and GATK. Variants were annotated for presence in the Single Nucleotide Polymorphism database (dbSNP138) and the National Heart, Lung, and Blood Institute exome sequencing project (ESP6500, Exome Variant Server, NHLBI GO Exome Sequencing Project (ESP) (http://evs.gs.washington.edu/EVS/) and those not leading to a change in protein sequence were filtered. KNSTRN variants were also evaluated using PolyPhen and SIFT and were queried in the COSMIC database v.64. CNV Analysis was performed using BIC-seq (as part of in-house pipeline Cancerscope) with default parameters and CNVs with $\log_{10}$ (p-value) < -200 were filtered out of the analysis.

Cell Culture and Chromosomal Spreads

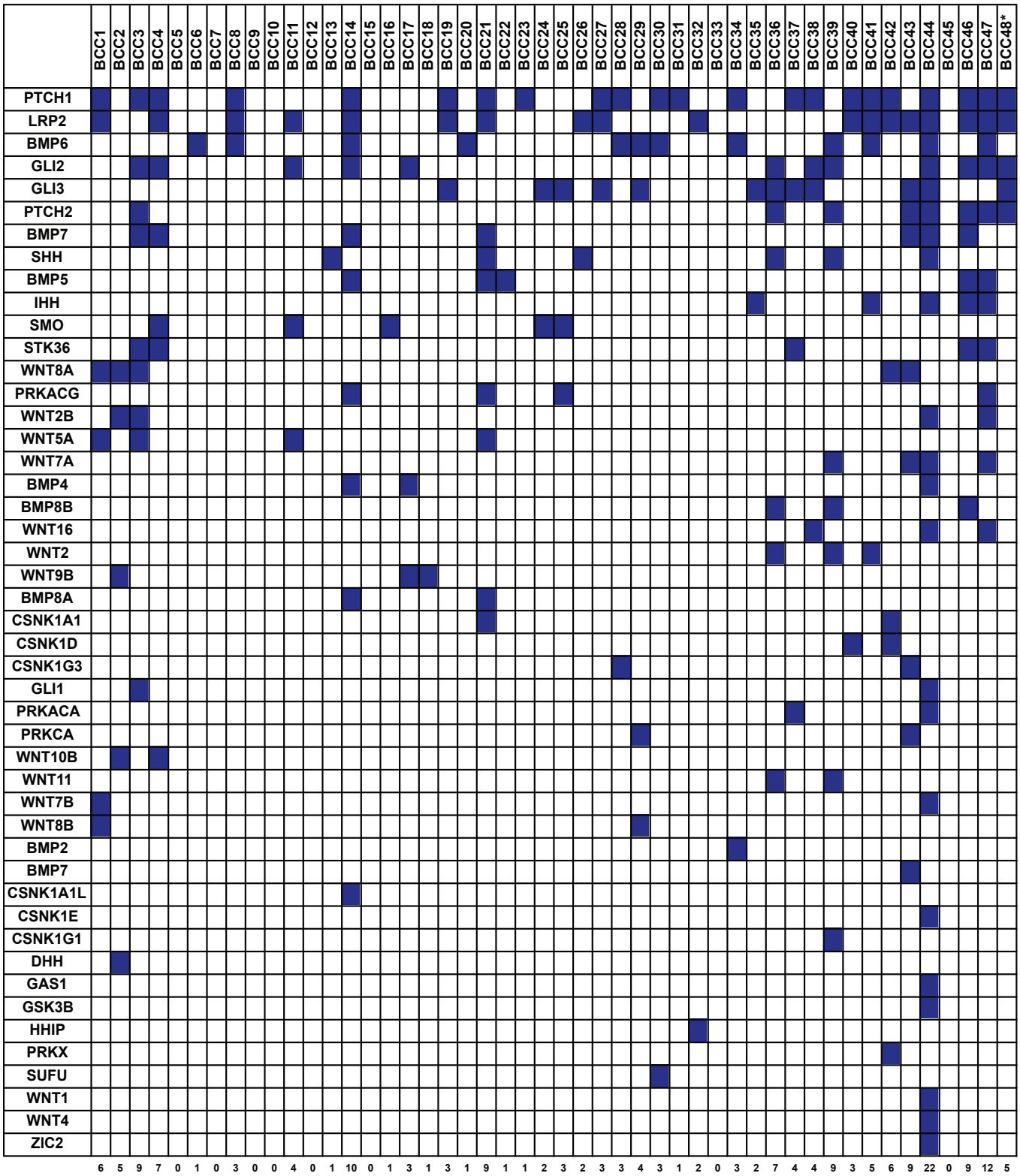
The ASZ001 murine BCC cell line was grown in 154CF (Invitrogen) supplemented with Pen/Strep, 2% chelexed FBS, and 0.05mM calcium, at 37°C in 5% CO2. ASZ001 cells were negative for mycoplasma using the MycoAlert Detection Kit (Lonza). Cells were transduced with 80ug/ml polybrene to express wild-type or S24F KNSTRN, which was generated through

site-directed mutagenesis and cloned into the pLEX lentiviral backbone for transduction. Transduced ASZ001 cells were plated at 70% confluence and treated with 500 ng/ml nocodazole (Sigma) for 18 h. Mitotic cells were collected by shake-off. Cells were resuspended in 75 mM KCl and cytospun onto glass coverslips with a cytocentrifuge (Shandon Cytospin 4). The resulting spreads were washed with KCM buffer (120 mM KCl, 20 mM NaCl, 10 mM Tris-HCl (pH 8.0), 0.5 mM EDTA and 0.1% Triton X-100), permeabilized in KCM buffer with 0.5% Triton X-100, stained with CENP-A antibody (Cell Signaling, C51A7, 1:800), fixed in 3.7% formaldehyde in KCM and stained with Hoechst (10 μg/ml). Images were collected in a z stack using an Olympus IX70 microscope and Softworx software (Applied Precision). The final images shown are maximum-intensity projections of deconvolved z stacks.

Statistics

A two-tailed, unpaired *t*-test with Welch's correction was performed to compare mean values between experimental groups in the functional assays. A two-tailed, unpaired *t*-test with Welch's correction was also used to compare mean exonic mutations, mean percent transitions, mean number of HH pathway mutations, and mean percent of the exome affected by CNVs between KNSTRN-mutated and KNSTRN-wildtype advanced BCCs. A Fisher's exact test was used to compare the difference in proportions for SMO, TP53 and PTCH1 mutations among KNSTRN-mutate and KNSTRN-wildtype advanced BCCs.

Supplemental Figure



Supplemental Table

	KNSTRN-Mutated Advanced BCCs (N = 4)	KNSTRN-WT Advanced BCCs (N = 14)
Total # Exonic Mutations (mean ± s.d.)	1299.3 ± 720.5	1455.3 ± 1285.6
Total # HH Pathway Mutations (mean ± s.d.)	8.5 ± 9.0	6.6 ± 3.4
% Transitions (mean ± s.d.)	85 ± 4.9	64.2 ± 21.5
% Samples with SMO Mutation	25	21.43
% Samples with TP53 Mutation	75	28.6
% Samples with PTCH1 Mutation	75	50